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## MYCOPESTICIDES

2        This application is a divisional of U.S. Application No. 09/678,141, filed  
3        10/04/2000, herein incorporated by reference.

### 4        BACKGROUND OF THE INVENTION

#### 5        1. Field of the Invention

6        The present invention relates to the use of fungal mycelium as a  
7        biopesticide. More particularly, the invention relates to the control and  
8        destruction of insects, including carpenter ants, fire ants, termites, flies,  
9        beetles, cockroaches and other pests, using fungal mycelia as both attractant  
10      and infectious agent.

#### 11      2. Description of the Related Art

12      The use of chemical pesticides is the cause of many secondary  
13      environmental problems aside from the death of the targeted pest. Poisoning  
14      of soil and underlying aquifers may occur, along with pollution of surface  
15      waters as a result of runoff. Increases in cancer, allergies, immune disorders,  
16      neurological diseases and even death in agricultural workers and consumers  
17      have been attributable to the use of pesticides. Chemical pesticides are  
18      increasingly regulated and even banned as a health risk to citizens.

19      Communities are increasingly in need of natural solutions to pest problems.

20      Compounding these problems, many pest type or vermin insects have  
21      developed a broad spectrum of resistance to chemical pesticides, resulting in

1 few commercially available pesticides that are effective without thorough and  
2 repeated applications. In addition to being largely ineffective and difficult  
3 and costly to apply, chemical pesticides present the further disadvantage of  
4 detrimental effects on non-target species, resulting in secondary pest  
5 outbreaks. It is believed that widespread use of broad-spectrum insecticides  
6 often destroys or greatly hampers the natural enemies of pest species, and  
7 pest species reinfest the area faster than non-target species, thereby allowing  
8 and encouraging further pest outbreaks. There is therefore a particular need  
9 for natural alternatives.

10 Biological control agents have been tried with varying results.  
11 Bacteria such as *Bacillus thuringiensis* are used with some success as a spray  
12 on plants susceptible to infestation with certain insects. Fungal control  
13 agents are another promising group of insect pathogens suitable for use as  
14 biopesticides for the control of insects. However, limited availability, cost and  
15 reliability have hampered the development of such fungal control agents.  
16 Host range and specificity has been a problem as well as an advantage; a  
17 fungal pathogen that is virulent and pathogenic to one insect species may be  
18 ineffective against other species, even those of the same genus. However,  
19 some success has been demonstrated.

20 The typical lifecycle of a pathogenic fungi control agent involves  
21 adhesions of the spore(s) to the host insect cuticle, spore germination and

1 penetration of the cuticle prior to growth in the hemocoel, death, saprophytic  
2 feeding and hyphal reemergence and sporulation. For example, U.S. patent  
3 no. 4,925,663 (1990) to Stimac discloses *Beauveria bassiana* used to control  
4 fire ants (*Solenopsis*). Rice, mycelia and spores (conidia) mixture may be  
5 applied to fire ants or used as a bait and carried down into the nest, thereby  
6 introducing spores. U.S. patent no. 4,942,030 (1990) to Osborne discloses  
7 control of whiteflies and other pests with *Paecilomyces fumosoroseus* Apopka  
8 spore conidia formulations. The *Paecilomyces* fungus is also useful for control  
9 of Diptera, Hymenoptera, Lepidoptera, Bemisia, Diaulurodes, Thrips,  
10 Spodoptera (beet army worm), Leptinotarsa (Colorado potato beetle),  
11 Lymantria (Gypsy moth), Tetranychus, Frankliniella, Echinothrips,  
12 Planococcus (Citrus mealybug) and Phenaococcus (Solanum mealybug). U.S.  
13 patent no. 5,165,929 (1992) to Howell discloses use of *Rhizopus nigricans* and  
14 other fungus in the order Mucorales as a fungal ant killer. U.S. patent no.  
15 5,413,784 (1995) to Wright *et al.* discloses compositions and processes  
16 directed to the use of *Beauveria bassiana* to control boll weevils, sweet potato  
17 whiteflies and cotton fleahoppers. U.S. patent no. 5,683,689 (1997) to Stimac  
18 *et al.* discloses conidial control of cockroaches, carpenter ants, and pharaoh  
19 ants using strains of *Beauveria bassiana* grown on rice. U.S. patent no.  
20 5,728,573 (1998) to Sugiura et al. discloses germinated fungi and rested spore  
21 termiticides of entomogenous fungus such as *Beauveria brongniartii*,

1    *Beauveria bassiana*, *Beauveria amorpha*, *Metarhizium anisopliae* and  
2    *Verticillium lecanii* for use against insects such as termites, cockroaches,  
3    ants, pill wood lice, sow bugs, large centipedes, and shield centipedes. U.S.  
4    patent no. 5,989,898 (1999) to Jin *et al.* is directed to packaged fungal  
5    conidia, particularly *Metarhizium* and *Beauveria*. The scientific journal  
6    literature also discusses similar uses of conidial preparations.

7           One disadvantage to such approaches is that the fungal lifecycle may  
8    be particularly sensitive to and dependent upon conditions of humidity,  
9    moisture and free water, particularly during the stages of germination,  
10   penetration of the cuticle prior to growth, and hyphal reemergence and  
11   sporulation after death of the insect.

12           Another continuing problem with existing techniques has been  
13   inconsistent bait acceptance. Baits are often bypassed and left uneaten. Such  
14   may be a particular problem with insects such as termites, as opposed to  
15   house ants and cockroaches, because it is usually not possible to remove  
16   competing food sources for termites. Attractants and feeding stimulants have  
17   sometimes increased the consistency of bait acceptance, but such increases  
18   cost and complexity, and there remains a continuing need for improved baits  
19   with improved bait acceptance.

20           A particular disadvantage with conidial fungal insect preparations  
21   becomes apparent from U.S. patent no. 5,595,746 (1997) to Milner *et al.* for

1 termite control. *Metarhizium anisopliae* conidia are disclosed and claimed as  
2 a termite repellent in uninfested areas and as a termite control method in  
3 infested areas. The difficulties of utilizing conidia or conidia/mycelium as a  
4 bait and/or contact insecticide are readily apparent when considering that  
5 conidia are effective as an insect repellent to termites and are repellent in  
6 varying degrees to most or all targeted insect pests. A repellent, of course,  
7 does not facilitate use as a bait or contact insecticide. This may be a factor in  
8 explaining why fungal insecticides have all too often proven more effective in  
9 the laboratory, where conidia may be unavoidable in the testing chamber or  
10 even directly applied to insects, than in the field.

11 U.S. patent no. 4,363,798 (1982) to D'Orazio is for termite baits  
12 utilizing brown rot fungus as an attractant and toxicant boron compounds in  
13 mixtures effectively sufficient to kill termites without creating bait shyness.  
14 Brown-rot inoculated wood is ground and mixed with cellulosic binder and  
15 boron compounds. Such an approach has the disadvantage of utilizing toxic  
16 boron compounds. In addition, the cultured mycelium must be further  
17 processed.

18 There is, therefore, a continuing need for enhancing the effectiveness of  
19 entomopathogenic (capable of causing insect disease) fungal products and  
20 methods. There is also a need for enhancing the attractiveness of such fungal  
21 pesticides to insects. There is also a need for improved packaging, shipping

1 and delivery methods.

2 In view of the foregoing disadvantages inherent in the known types of  
3 fungal biocontrol agents, the present invention provides improved fungal  
4 biocontrol agents and methods of using such agents.

5

## 6 SUMMARY OF THE INVENTION

7 The present invention offers an environmentally benign approach to  
8 insect control by attracting the insects who ingest latent preconidial  
9 mycelium (which may be fresh, dried or freeze-dried) which then infects the  
10 host. The preconidial mycelium is both the attractant and the pathogenic  
11 agent. The infected insects carrying the fungal hyphae become a vector back  
12 to the central colony, further dispersing the fungal pathogen. Mycelium is  
13 grown in pure culture using standard fermentation techniques for in vitro  
14 propagation. The fermented mycelia is diluted and transferred into a  
15 sterilized grain or a mixture of sterilized grains. Once inoculated, the  
16 fermented mycelia matures to a state prior to conidia formation. The  
17 preconidial mycelium may be utilized as is or may be arrested in its  
18 development through flash chilling (or by other means such as air-drying or  
19 refrigeration) and packaged in spoilage-proof or sealed packages. The end-  
20 user facilitates opening the package and placing the exposed mycelia-grain  
21 contents in the vicinity of recent pest activity.

1        The present invention thus provides improved products and methods  
2    wherein the fungal mycelium acts as bait and attractant and as an ingested  
3    or food insecticide, palatable enough that insects will readily consume it even  
4    in the presence of competing food sources, with high recruitment of other  
5    insects among social insects that exhibit such behavior. This results in  
6    multiple visits to a highly attractive pathogenic bait, thereby providing  
7    effective individual insect and/or colony inoculation.

8        The present invention further provides these and other advantages  
9    with improved control of insect pests using fungal insecticidal compositions  
10   (mycopesticides) having strong attractant properties and placing these  
11   attractant mycopesticides in or around an object or area to be protected. The  
12   present invention also provides insecticidal baits which use, as a toxicant,  
13   relatively innocuous, naturally occurring materials as the active agent, so as  
14   to control insects without undue effect on the ecology. Finally, by actively  
15   avoiding the use of conidia, the time and expense of raising conidial stage  
16   mycelium and/or separating conidia is avoided.

17        Still further objects and advantages of the present invention will  
18   become more apparent from the following detailed description and appended  
19   claims.

20        Before explaining the disclosed embodiments of the present invention  
21   in detail, it is to be understood that the invention is not limited in its

1 application to the details of the particular products and methods illustrated,  
2 since the invention is capable of other embodiments. Also, the terminology  
3 used herein is for the purpose of description and not of limitation.

4

5 **DESCRIPTION OF THE PREFERRED EMBODIMENTS**

6 The present invention provides improved mycopesticides (fungal  
7 mycelia utilized as insect biopesticides). The attractiveness of fungal mycelia  
8 to many species is well known. Black Angus cows have been observed  
9 running uphill (a rare event) to reach spent Oyster mushroom mycelium on  
10 straw. Cultured mycelia such as Morel mycelium is considered a delicacy  
11 when added to human foods; gourmet mushrooms themselves are a form of  
12 mycelium fruitbody. Indeed, the attractiveness of mycelial scents is to a  
13 great degree responsible for the fresh and refreshing scent of a forest after a  
14 rain, a result of the mushroom mycelia responding to the humid conditions  
15 with rapid growth. Mycelium is also known to be highly attractive to insects.  
16 Certain ants, termites and wood-boring beetles are known to cultivate and  
17 raise fungal mycelium as an exclusive food source ("ambrosia fungi") and  
18 mycelium is a preferred food source of many insect species. As discussed  
19 above, brown rot mycelium (the mycelial stage of a wood-rotting type of  
20 fungus that produces polypore mushrooms) has been used as an attractant  
21 for termites.

1           However, for insect control typical use of fungal pathogens has  
2   involved use of either conidia (spores) or a mixture of conidia and mycelium  
3   as a “contact insecticide” control agent. Such conidial contact insecticides  
4   suffer two major disadvantages: 1) conidia and conidia/mycelium  
5   preparations are to some degree unattractive or even repellent to insects; and  
6   2) such conidia preparations are highly dependent on free water or humid  
7   conditions for gestation and infestation during the typical life cycle of an  
8   insect fungal control agent. Furthermore, conidia have been found to be more  
9   effective against “stressed” insects and/or insect populations than against  
10   healthy insects and populations. For these and other reasons, conidia of  
11   entomopathogenic fungi have often been much more effective under  
12   laboratory conditions than in the field.

13           Noting that conidia have been utilized as a repellent for termites,  
14   further investigation of the preconidial and conidial stages were undertaken.  
15   The preconidial stage is the vegetative stage of the fungus, prior to the  
16   formation of structures leading to the release of air-borne spores (which is  
17   distinguished from fragmentation of hyphae which can become airborne if  
18   dried). Those skilled in the art will recognize that mycelia or mycelial hyphal  
19   fragments may form structures such as arthrospores (a preconidial structure  
20   imbedded within the mycelia) and such should be considered a “preconidial  
21   mycelium” as discussed elsewhere. It was found that the “fragrance

1   signature" of the mycelium is a strong attractant to insects, but only prior to  
2   conidia formation. After conidia formulation, the conidia/mycelium was  
3   found to be repellent to insects such as carpenter ants. The odor was found  
4   to be similarly pleasing to humans when preconidial and repellent when post-  
5   conidial. It was noted such fragrance signatures are "washed away" or lost  
6   when mycelium is grown via liquid fermentation. It was also noted liquid  
7   fermentation utilizing a typical fermentor with bubbled air mixing will  
8   promote conidia formation, with such conidia production being even further  
9   promoted by the common commercial practice of utilizing bubbled oxygen.

10       It was further found that fungal control agents are much more effective  
11   when preconidial mycopicidical mycelium is ingested by the targeted insect  
12   as compared to conidia or post-conidial mycelium/conidia offered to targeted  
13   insects for the purpose of infection by contact. Whereas conidia have little or  
14   no effect by ingestion or vapor, preconidial mycelium has proven to be highly  
15   effective by ingestion, the mycelial hyphae already being in a state of active  
16   growth when ingested. Furthermore, whereas conidial preparations are more  
17   dependent upon humidity in the insect environments, a preconidial  
18   mycopicidical mycelium which is eaten by an insect is dependent upon  
19   humidity only in the immediate vicinity of the mycelium, the humidity of the  
20   mycelium of course being much more easily controlled than in the wider  
21   general insect environment.

1        It has further been found that the preconidial stage can be maintained  
2    provided carbon dioxide (CO<sub>2</sub>) levels are maintained at an elevated level. The  
3    CO<sub>2</sub> levels preferably range from 2,000-200,000 ppm, more preferably in the  
4    range of 10,000-50,000+ ppm. Once exposed to fresh air, the mycelium can  
5    produce conidia in just a few days. By preventing conidial formation, the  
6    mycelium continues to accumulate mycelial biomass (sans conidia). Even  
7    after maturation, the mycopesticidal mycelium may be maintained in a  
8    preconidial state via elevated carbon dioxide levels. This prevention of  
9    conidia formation is an active component in this technology, as conidia  
10   formation is actively avoided.

11        Mycopesticidal mycelium is grown in pure culture using standard  
12   fermentation techniques well established for in vitro propagation. The  
13   fermented mycelia is diluted and transferred into a sterilized grain or a  
14   mixture of sterilized grains (rice, wheat, rye, oat, millet, sorghum, corn,  
15   barley, etc. The grain is pressure steam-sterilized at 1 kg/cm<sup>2</sup> (15 psi) for  
16   several hours. The master broth is transferred aseptically manually or by  
17   using peristaltic pumps into the sterilized grain. Growth mediums  
18   containing sawdust, sugar cane, corn cobs, cardboard, paper or other  
19   substances containing cellulose may be utilized for cellulose loving insects  
20   such as termites if desired. A variety of containers are used for incubation,  
21   including high-density polyethylene and polypropylene bags, glass and

1 polypropylene jars, metal containers, etc.). Use of such containers provides a  
2 convenient method of maintaining high CO<sub>2</sub> levels, as the growing mycelium  
3 gives off carbon dioxide. CO<sub>2</sub> levels will rise to acceptable levels for use in the  
4 present invention even if filter patches, disks or materials are utilized to  
5 allow some gas exchange. Alternatively, grow rooms may be maintained at  
6 high CO<sub>2</sub> levels. Further information on such culture techniques may be  
7 found in the applicant's books, *Growing Gourmet and Medicinal Mushrooms*  
8 (1993, 2000) and *The Mushroom Cultivator* (1983) (with J. Chilton), and in  
9 standard microbiology manuals.

10 Once inoculated, the mycelia on grain matures to a state prior to  
11 conidia formation and may be utilized fresh or metabolically arrested or  
12 developmentally arrested through flash chilling (freeze-drying), drying,  
13 refrigeration or by other means. It will be understood that such metabolic  
14 arresting of development may encompass either a slowing of metabolism and  
15 development (such as refrigeration) or a total suspension or shutdown of  
16 metabolism (freeze-drying, air-drying and cryogenic suspension). When  
17 freeze-drying, drying or other known methods of arresting development are  
18 utilized, it is essential that freeze-drying or other methods occur at an early  
19 stage in the life cycle of these fungi before the repellent spores are produced.  
20 The mycelium-impregnated grain media may then be fragmented and packed  
21 in appropriate containers for commerce. Fresh mycelium may be shipped in

1 growing containers such as jars or spawn bags, which allows easy  
2 maintenance of a high carbon dioxide atmosphere and maintenance of sterile  
3 conditions during shipping. It is preferable that the mycelium be utilized or  
4 processed while vigorous, before it “over-matures” and becomes less viable for  
5 lack of new food to digest and accumulation of waste products.

6 When the freeze-dried or dried mycelium is reactivated via  
7 rehydration, the mycelium is preferably allowed to slowly rehydrate through  
8 controlled absorption of atmospheric humidity, with the result that the  
9 mycelium “wakes up” and wicks into the air. This is a totally different  
10 response from immersion, which often results in bacterial contamination and  
11 souring, as the freeze-dried mycelium suffers when immersed in water. Such  
12 rehydration and reactivation may be carried out on a large scale through high  
13 humidity atmosphere, or may be accomplished by an end user through use of  
14 wet materials such as sponges, wicking materials and/or other evaporative  
15 materials or by atmospheric absorption of humidity from a remote water  
16 reservoir. Such end user rehydration may be carried out in any suitable  
17 container or a bait box if desired. Warming is suitable for reactivation of  
18 refrigerated materials; it is preferred that the mycelium not be refrigerated  
19 for extended lengths of time.

20 Novel features of the invention include the use of a vector of  
21 parasitization that relies on hyphal fragments, not spores or conidia; the use

1 of a single mycelium as both attractant fungus and pathogen; the use of high  
2 levels of CO<sub>2</sub> to grow and maintain preconidial mycelium; and the preferred  
3 use of various methods to arrest development at the preconidial stage to  
4 facilitate growth, packaging, shipping and convenient application by an end  
5 user. More than one fungus can be used to create a matrix of characteristics  
6 to increase usefulness as a natural pesticide.

7 In general, preferred mycopesticidal species as pathogens are  
8 somewhat slow-acting (that is, not immediately fatal), so as to avoid bait  
9 shyness and to avoid learning effects in social insects before individuals have  
10 distributed mycelium to other members of the colony. In many applications it  
11 may be preferable to utilize a mixture or matrix of several species of  
12 entomopathogenic fungus with different characteristics, maturation and  
13 growth rates, preferred conditions, virulence and pathogenicity, time to insect  
14 death, etc., while in other applications a single species may be preferred.

15 Similarly, with reference to a single species, a mixture of strains or a single  
16 strain may be utilized. Those skilled in the art will recognize that such  
17 characteristics can be selected for utilizing known techniques and bioassays.

18 The mycopesticides disclosed herein may also be optionally enhanced by the  
19 use of other baits, attractants, arrestants, feeding stimulants, sex  
20 pheromones, aggregating pheromones, trail pheromones, etc.

21 There are numerous entomogenous and entomopathogenic fungal

1 species known. Those skilled in the art will recognize that the above  
2 preconidial fungi methods and products may be favorably applied to all such  
3 insecticidal fungal species, and it is the intent of the inventor that the  
4 invention be understood to cover such. Suitable entomopathogenic fungi  
5 include *Metarhizium*, *Beauveria*, *Paecilomyces*, *Hirsutella*, *Verticillium* and  
6 other fungi imperfecti, the Entomophthoraceae and other Phycomycetes, and  
7 sexually reproducing fungi such as *Cordyceps* and other Ascomycetes.

8 By way of example, but not of limitation, preferred mycopesticides  
9 include *Metarhizium anisopliae* ("green muscarine" for pests such as  
10 carpenter ants, including *Camponotus modoc*, *C. vicinus*, *C. ferrugineus*, *C.*  
11 *floridanus*, *C. pennsylvanicus*, *C. herculeanus*, *C. varigatus* and *C. vicinus*,  
12 fire ants (*Solenopsis invicta* and *Solenopsis richteri*), termites, including  
13 *Coptotermes*, *Reticulitermes*, *Cryptotermes*, *Incisitermes*, *Macrotermes* and  
14 *Odontotermes*, pasture scarabs such as *Adoryphorus couloni*, spittle bug  
15 *Mahanarva posticata*, corn earworm *Helicoverpa zea*, tobacco hornworm  
16 *Manduca sexta*, sugar cane froghopper, pill wood lice, sow bugs, large  
17 centipedes, shield centipedes, wheat cockchafer, beetle grubs, greenhouse  
18 pests such as Coleoptera and Lepidoptera, etc.); *Metarhizium flaviride*  
19 (grasshoppers and locusts); *Beauveria bassiana* ("white muscarine" for  
20 termites including Formosan termites, carpenter ants, fire ants, pharaoh  
21 ants, cockroaches, whiteflies, thrips, aphids, mealybugs, boll weevils, sweet

1 potato whiteflies, cotton fleahoppers, European and Asiatic corn borers and  
2 larvae of other Lepidoptera, codling moth, chinch bug, soft-bodied insects in  
3 the orders Homoptero and Coleoptera, Heteroptera, etc.); *Beauveria*  
4 *brongniartii* (white grubs and cockchafers, *Hoplochelis marginalis*,  
5 *Melolontha melontha*); *Paecilomyces fumosoroseus* (whiteflies, thrips, aphids,  
6 spider mites, mealybugs, beet army worm, Colorado potato beetle, Gypsy  
7 moth, etc.); *Verticillium lecanii* (greenhouse pests, whiteflies and aphids);  
8 *Hirsutella citriformis* (rice brown planthopper); *Hirsutella thompsoni* (citrus  
9 rust mite); and the wide variety of *Cordyceps* for baiting and killing pests  
10 such as beetles, flies, cockroaches, earwigs (*Forficula auricularia*), carpenter  
11 ants and various other insect pests, including *Cordyceps variabilis*, including  
12 imperfect forms (fly larvae, Xylophagidae family of the Diptera order),  
13 *Cordyceps facis* and *C. subsessilis*, (beetle larvae in the coleopteran family,  
14 Scarabaeidae), *Cordyceps myrmecophila* (ants); *Cordyceps sphecocephala*  
15 (wasps), *Cordyceps entomorrhiza* (beetle larvae), *Cordyceps gracilis* (larvae of  
16 beetles and moths), *Cordyceps militaris*, *Cordyceps washingtonensis*,  
17 *Cordyceps melolanthae* (beetles and beetle grubs), *Cordyceps ravenelii* (beetle  
18 grubs), *Cordyceps unilateralis* (ants, carpenter ants, bees and wasps) and  
19 *Cordyceps clavulata* (scale insects).  
20 With regard to the sexually reproducing *Cordyceps*, preconidial or pre-  
21 sporulation refers to the pre-fruiting state. The term “preconidial” has a

1 somewhat different meaning than with most other entomopathogenic fungi,  
2 as *Cordyceps* is a “fungi perfecti” or mushroom fungi, whereas the other non-  
3 mushroom fungi referred to herein are the more primitive “fungi imperfecti.”  
4 Some or all *Cordyceps* fungi are believed to be anamorphic or dimorphic and  
5 have conidial stages within the imperfect fungal genera including *Beauveria*,  
6 *Metarhizium*, *Paecilomyces*, *Hirsutella*, *Verticillium*, *Aspergillus*,  
7 *Akanthomyces*, *Desmidiospora*, *Hymenostilbe*, *Mariannaea*, *Nomuraea*,  
8 *Paraisaria*, *Tolypocladium*, *Spicaria* (=*Isaria*) and *Botrytis*. For example, *C.*  
9 *subsessilis* is the perfect form of *Tolypocladium inflatum*, an anamorph  
10 (imperfect) form which produces cyclosporin. Hodge et al., *Tolypocladium*  
11 *inflatum* is the anamorph of *Cordyceps subsessilis*. *Mycologia* 88(5): 715-719  
12 (1996). *Cordyceps militaris* (Fr.) Lk. is also thought to be dimorphic, the  
13 conidial stage of which is believed to be a *Cephalosporium*. DNA studies are  
14 expected to better elucidate these relationships. As a further complexity, in  
15 addition to possible anamorphs and dimorphs, *Cordyceps* species also  
16 demonstrate nonsexual imperfect stages of development. As used herein,  
17 unless otherwise specified, preconidial *Cordyceps* refers to the pre-sporulation  
18 mycelial stage of the *Cordyceps* mushrooms, including any preconidial  
19 imperfect stages, but not any conidia bearing imperfect stages.  
20 For initial experimentation, a *Metarhizium anisopliae* from naturally  
21 occurring sources and the carpenter ant were selected. *M. anisopliae* was

1 obtained from a public culture collection and used without further selection  
2 for virulence and/or pathogenicity; a publicly available strain free of  
3 proprietary or patent restrictions on use was selected as offering a preferred  
4 source and a more demanding initial test than strains selected for specific  
5 pathogenicity. It will be understood, of course, that strains selected for  
6 specific characteristics and pathogenicity against specific insects will in  
7 general offer the best mode of practicing the invention. The carpenter ant  
8 offered several advantages: ants are typically more resistant to spores than  
9 termites and other insects, carpenter ants are a very destructive pest, the  
10 effect on other ant species could also be viewed, and the applicant enjoyed  
11 easy access to an experimental site as his residence was in danger of collapse  
12 due to long term structural infestation by carpenter ants.

### EXAMPLE 1

14        *Metarhizium anisopliae* was grown in pure culture using standard  
15    fermentation techniques and diluted and aseptically transferred to grain  
16    (rice) which had been pressure steam-sterilized at 1 kg/cm<sup>2</sup> (15 psi). The  
17    fermented mycelia matured to a state prior to conidia formation and the  
18    fungus colonized grain was offered at the site of debris piles caused by  
19    carpenter ants at the 1,100-1,200 sq. ft. house of the applicant's residence  
20    located in Shelton, Washington, U.S.A. Approximately 10-20 grams of  
21    preconidial mycelium of *Metarhizium anisopliae*, grown on autoclaved rice

1 and having been incubated for two weeks, was presented at the location of  
2 debris piles next to the interior face of an exterior wall within the house. The  
3 non-sporulating mycelium was presented on a dollhouse dinner dish and left  
4 exposed to the air. Later that night, the applicants' daughter urgently awoke  
5 the applicant when she observed carpenter ants feasting en masse on the  
6 non-sporulating mycelium of the presented *Metarhizium*. The applicant and  
7 his family observed approximately a dozen carpenter ants ingesting  
8 mycelium and retreating into the wall, carrying the infectious mycelium with  
9 them. In a week's time, the carpenter ant colony became inactive, killing the  
10 nest of ants, and no evidence of carpenter ant activity was observed  
11 henceforth, saving the structure from further structural damage. Months  
12 later, the ecological niche once occupied by the carpenter ants was taken over  
13 by common household Sugar and Honey ants which were unaffected by the  
14 *Metarhizium anisopliae*.

### EXAMPLE 2

16           Cultivate strains of *Metarhizium*, *Beauveria* and *Cordyceps* on grain as  
17   above under high CO<sub>2</sub> conditions to produce preconidial mycelium. Freeze-  
18   dry and rehydrate. Apply as bait and pathogen at locations infested by  
19   insects such as carpenter ants, termites, beetles, flies, fire ants, cockroaches  
20   and other insect pests and vermin.

### EXAMPLE 3

1 Drill one or more holes into a termite colony mound or tree mound.

2 Insert entomopathogenic preconidial mycopesticidal mycelium into the holes.

3 Cover the holes to prevent entry of marauding ants.

4 No limitations with respect to the specific embodiments disclosed

5 herein is intended or should be inferred. While preferred embodiments of the

6 present invention have been shown and described, it will be apparent to those

7 skilled in the art that many changes and modifications may be made without

8 departing from the invention in its broader aspects. The appended claims are

9 therefore intended to cover all such changes and modifications as fall within

10 the true spirit and scope of the invention.

11